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Synthesis, characterization and evaluation of antibacterial & anti-fungal activity of 2, 5-disubstituted 1, 3, 4-oxadiazole derivatives

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Abstract
A new series of some pyridine condensed oxadiazole derivatives were prepared by reacting pyridine derivative with various aromatic carboxylic acids. In the present work 9 different 2, 5-disubstituted 1, 3, 4 oxadiazole derivatives (3a-i) were synthesized. Pyridine 4-carboxylic acid (Nicotinic acid) is converted into methyl pyridine 4-carboxlate by esterification. Pyridine 4-carboxlate is converted to pyridine 4-carbohydrazide (Isoniazid) by treating with hydrazine hydrate. Pyridine 4-carbohydrazide is converted to 2, 5-disubstituted 1, 3, 4 oxadiazole derivatives by treating with different types of aromatic carboxylic acids in the presence of $POCl_3$**.** Confirmation of the chemical structure of the synthesized compounds was substantiated by TLC, IR, ^{1}H NMR, and MS spectroscopy.
All the synthesized compounds were tested for in-vitro antibacterial (against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*) and antifungal activity (against *Aspergillus niger*). 5e, 5i, 5b, 5d showed better inhibition as compared to the standard Ciprofloxacin and 5b, 5d, 5e & 5i showed good inhibition as compared to the standard Ketokonazole.

Keywords: Oxadiazole, *in-vitro* antibacterial, antifungal, standards.

Introduction
Heterocyclic by far are the largest classical divisions of organic chemistry and are of immense importance in biologically and industrially. The chemistry of heterocyclic compounds has been an interesting field of study for a long time. The synthesis of novel Oxadiazole derivatives and investigation of their chemical and biological behaviour have gained more importance in recent decades for biological, medical and agricultural reasons. 1, 3, 4-Oxadiazoles belongs to a group of heterocyclic compounds that exhibit a wide range of biological activities [1]. A lot of compounds containing such an arrangement demonstrate strong antibacterial, anticonvulsant and anticancer activities, some of them are even used to fight infections involving AIDS [2-4]. They also have some industrial applications in agriculture as pesticides, acaricides and nematodes [5, 6] or in material science because of their precious electrochemical properties [7, 8].
The most popular method to synthesize 1,3,4-oxadiazoles uses acid hydrazides as substrates that undergo reaction with aromatic aldehydes [9], carboxylic acids [4] and orthoesters [10]. Another comprises the reactions of diacyl hydrazines with a range of cyclo dehydrating agents, for example: polyphosphoric acid [11]. Phosphorus oxychloride [12], thionyl chloride [13] or boron trifluoride diethyl etherate [14].
Certain oxadiazoles compounds can reveal liquid crystalline features and in particular, as recently demonstrated can show the elusive biaxial nematic phase. Theoretically proposed in 1970 by Freiser, the biaxiality can be observed in the nematic phase of boomerang-shaped oxadiazoles [15-17]. In general, the oxadiazoles are interesting for applications to electroluminescent devices where they are the emissive materials [18]. It is worthy to mention that this substitution formulated a unique structure, with potent biological activities.

Experimental
Melting points of the synthesized compounds were determined in open capillary tubes and were uncorrected. IR spectra were recorded on Shimadzu FTIR Spectrophotometer with KBr pellets. Mass Spectra were recorded on GCMS QD 5000 Shimadzu. H1NMR Spectra was recorded on Bruker AV- 500 MHz, using DMSO as solvent. The test compounds were synthesized by the following procedure.

Synthesis of methyl-pyridine- 4-carboxylate: (1)
Methyl-pyridine- 4-carboxylate was prepared by dissolving 0.01mole of nicotinic acid (pyridine- 4- carboxylic acid) in 35ml of methanol. Carboxylic acid group is esterified with methanol, the reaction is processed by refluxing the mixture for 5hours by adding few drops of $H_2SO_{4\ as}$ catalyst. The final product was obtained as a no aqueous liquid which was continued for the next step for the preparation of pyridine – 4 – carbohydrazide.

Synthesis of pyridine – 4 – carbohydrazide (Isoniazid): (2)
The mixture of compound1 (o.o1mol) and 4ml 99%hydrazine hydrate was refluxed for 6hours.The reaction mixture was then cooled and evaporated. The solid precipitate was obtained, dried and re crystallised from methanol.
Yield =70%; Melting point =220-221 ^{0}C.

Compound 3a

4-(5-phenyl-1, 3, 4-oxadizol-2-yl) pyridine

IR (KBr, cm-1): 2942.58 (Ar-C-H), 1552.92 (Ar-C=C), 3079.90 (=C-H), 1406.76 (Ar-C=N). ^{1}H-NMR (CDCl3 δ, ppm): 8.07 (m, 2H), 8.90 (o, 2H), 7.38 (o, 2H, Ar-H), 7.57(m, 2H, Ar-H), 8.13(p, 1H, Ar-H). MS 222.2 (M-).

Compound 3b

4-[5-(4-methoxyphenyl)-1, 3, 4- oxadizol-2-yl] pyridine

IR (KBr, cm-1): 2943.39 (Ar-C-H), 1608.82 (Ar-C=C), 3079.90 (=C-H), 1412.01 (Ar-C=N). ^{1}H-NMR (CDCl3 δ, ppm): 8.10 (m, 2H), 8.91 (o, 2H), 7.92 (o, 1H, Ar-H), 7.60(m, 1H, Ar-H), 7.14-7.25(p, 2H, Ar-H), 3.95(3H, aliphatic C – H). MS 254.3 (M+).

Compound 3c

4-[5-(4-cholorophenyl)-1, 3, 4- oxadizol-2-yl] pyridine

IR (KBr, cm-1): 2943.52 (Ar-C-H), 1602.34 (Ar-C=C), 3052.39 (=C-H), 1497.93 (Ar-C=N). ^{1}H-NMR (CDCl3 δ, ppm): 8.10 (m, 2H), 8.90 (o, 2H), 8.20 (o, 2H, Ar-H), 7.60(m, 2H, Ar-H).

Compound 3f

4-[5-(4-nitrophenyl)-1, 3, 4- oxadizol-2-yl] pyridine

IR (KBr, cm-1): 3279.31 (Ar-C-H), 1667.73 (Ar-C=C), 3177.90 (=C-H), 1459.73 (Ar-C=N) 1602.33(Ar – NO_2), 1643.23(O – N = O). ^{1}H-NMR (CDCl3 δ, ppm): 8.10 (m, 2H), 8.90 (o, 2H), 8.20 (o, 2H, Ar-H), 7.60(m, 2H, Ar-H).

Compound 3h

2-[5-(pyridine-4-yl)-1, 3, 4- oxadizol-2-yl] phenol

IR (KBr, cm-1): 3379.16 (Ar-C-H), 1682.59 (Ar-C=C), 3079.90 (=C-H), 1458.76 (Ar-C=N) 3385.34 (Ar – OH). ^{1}H-NMR (CDCl3 δ, ppm): 8.10 (m, 2H), 8.90 (o, 2H), 7.89 – 7.45 (2H, Ar-H), 7.11 – 7.06 (2H, Ar-H), 10.13 (1H, Ar – OH).

Synthesis of 2-(4-pyridine)-5-phenyl-1,3,4-oxadiazole:(3)

A solution of 0.01mol of compound 2 and equimolar amount of approximate benzoic acid was heated in the presence of $POCl_3$ under reflux for 8 hours. The precipitate was obtain and washed with water and cleaned with boiling ethanol and recrystallized from ethanol.

The same procedure was repeated by taking different aromatic acids of synthesized derivatives.

Scheme of Work

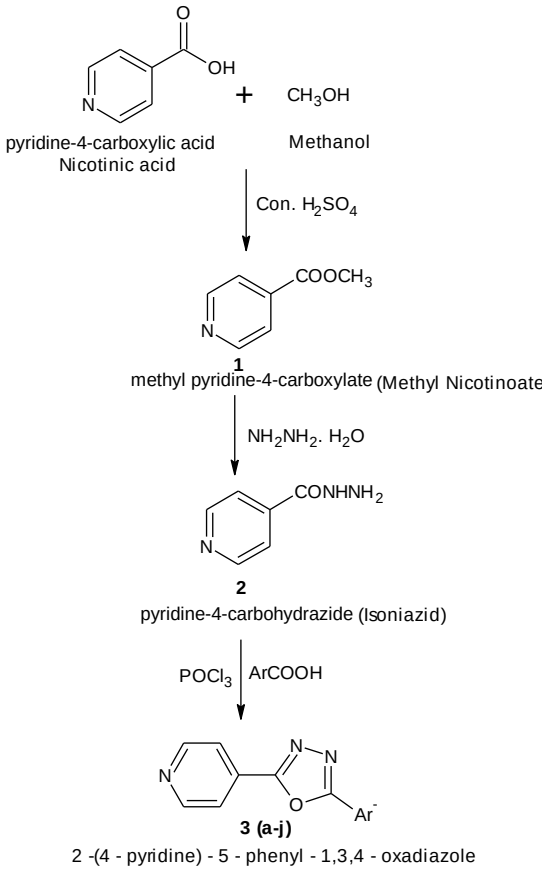


Table 1: physicochemical characteristic of intermediate compounds (3a-3i)

S.No	Compound code	Melting point (^{0}c)	Molecular formula	Molecular weight	Appearance	% yield
1	3a	90-96 ^{0}C	$C_{13}H_9N_3O$	223	Creamish	32%
2	3b	158-165 ^{0}C	$C_{14}H_{11}N_3O_2$	254	Light brown	79%
3	3c	118-122 ^{0}C	$C_{13}H_8N_3OCl$	258	Creamish	42%
4	3d	101-104 ^{0}C	$C_{14}H_{11}N_3O$	237	Reddish brown	6%
5	3e	148-154 ^{0}C	$C_{13}H_9N_3O_2$	239	Pale yellow	68%
6	3f	237-240 ^{0}C	$C_{13}H_8N_4O_3$	268	Black	89%
7	3g	175-180 ^{0}C	$C_{13}H_7N_5O_5$	313	Creamish yellow	79%
8	3h	78-82 ^{0}C	$C_{13}H_9N_3O_2$	239	Creamish yellow	92%
9	3i	102-105 ^{0}C	$C_{15}H_{11}N_3O_3$	281	yellow	28%

Antibacterial Activity

The synthesized compounds were screened for *In vitro* antimicrobial activity by turbid metric method. This method is used for determining the selective effectiveness of the antibacterial activity. The standard antibiotic selected for study of the antibacterial activity is *Ciprofloxacin*. In the present study the following bacteria were used. *Escherichia coli* (Gram – ve) (ATCC 700722), *Bacillus subtilis* (Gram + ve) (ATCC 14580), *Staphylococcus aureus* (Gram + ve) (ATCC 700294)

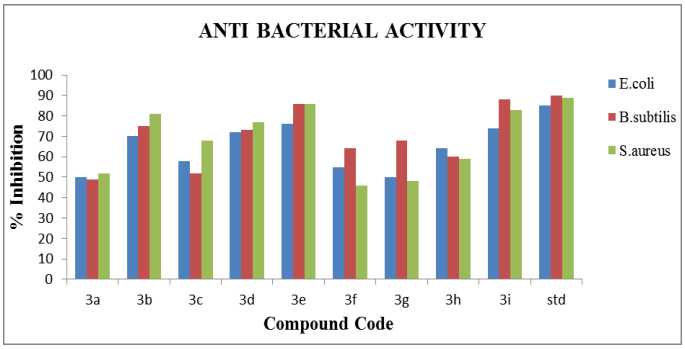
Each test compound (10 mg) is to be dissolved in dimethyl sulfoxide (10 ml) & diluted to give a concentration of 1,000µg/ml. It is to be serially diluted so as to give concentration of 50 µg/ml, 100µg/ml, 150 µg/ml, 200 µg/ml, 250 µg/ml, using DMSO as solvent. Percentage of inhibition was calculated according to the formula.

% Inhibition = 100(P-Q)/P

Where P=absorbance without test sample and Q=absorbance with test sample.

Table 2: % Inhibition of compounds 3a-3j against various bacteria

Compound Code	% Inhibition		
	E. coli	*B. subtilis*	*S. aureus*
3a	50	49	52
3b	70	75	81
3c	58	52	68
3d	72	73	77
3e	76	86	86
3f	55	64	46
3g	50	68	48
3h	64	60	59
3i	74	88	83
Standard	85	90	89



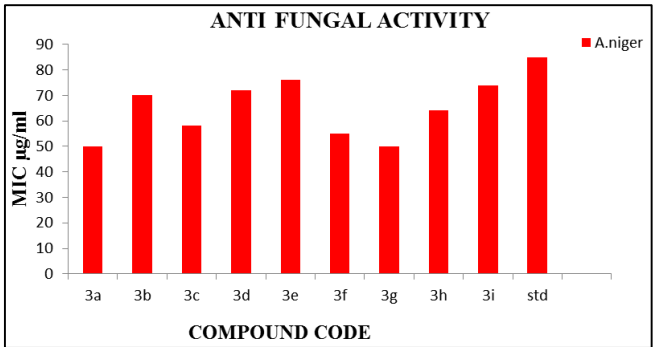
Anti Fungal Activity

The synthesized compounds were screened for *In vitro* antimicrobial activity by turbidimetric method. This method is used for determining the selective effectiveness of the antifungal activity. The standard antibiotic selected for study of the antifungal activity is *ketoconazole*. In the present study *Aspergillus niger* (ATCC 1015**)** were used.

The definite volume of Sabouraud dextrose broth (3gm) in 200ml of each distilled water and the pH was adjusted to 7.2. This solution was sterilized by autoclaving for 15-20 mins at 121 ^{0}C.One day prior to this testing, inoculation of the above fungal cultures were made in the potato dextrose broth incubated at 37 ^{0}C for 18-24 hrs. Each test compound (10mg) was dissolved in dimethyl sulfoxide (10ml) & diluted to give a concentration of 1,000µg/ml. It was serially diluted so as to give concentrations of 50µg/ml, 100µg/ml, 150µg/ml, 200µg/ml, 250µg/ml using DMSO as solvent. Percentage of inhibition was calculated according to the formula. % Inhibition = 100 (P-Q)/P

Table 3: Inhibition of compounds 3a-3j against *A.niger*

Compound Code	*A.niger*
3a	43
3b	80
3c	55
3d	74
3e	85
3f	62
3g	64
3h	40
3i	82
Standard	90



Result and Discussion

The anti-bacterial and anti-fungal activity of 2, 5-disubstituted 1, 3, 4 oxadiazole derivatives [19-22] were revealed from literature search [23]. Present study was designed to synthesize and evaluate anti bacterial activity and anti-fungal activity of several substituted 2, 5-disubstituted 1, 3, 4 oxadiazole derivatives. Antibacterial and antifungal activities were measured by turbidimetric method.% inhibition for antibacterial activity revealed that some of the test compounds like 3e, 3i, 3b,3d showed better inhibition as compared to the standard ciprofloxacin. against all the three bacterial stains *Escherichia coli*, *Bacillus subtilis and Staphylococcus aureus.* Antifungal screening revealed that the test compounds showed good to moderate activity against *Aspergillus niger*. % inhibition for antifungal activity revealed that some of the test compounds like 3b, 3d, 3e & 3i showed good inhibition as compared to the standard ketokonazole, due to substitution with OH, Cl and NO_2 groups. 5a & 5h showed moderately activity. The studies showed that 3i (-$OCOCH_3$), 3e (4-OH) and 3b (-OCH_3) showed potent anti-bacterial activity, compounds 3e (4-OH), 3b (-OCH_3) and 3i (-$OCOCH_3$) showed good anti-fungal activity. From our study we can conclude that 3e (4-OH), 3b (-OCH_3) and 3i (-$OCOCH_3$ can be considered as a lead among the series and bears a possibility to find a newer anti-bacterial and anti-fungal drug. So these entities could prove as better molecules in future.

Conclusion: A set of nine oxadiazole derivatives were designed and synthesized using appropriate synthetic Scheme. The synthesized compounds were purified and well characterized by TLC, IR, ^{1}HNMR, and GC-MS data. Compounds 3e, 3i, 3b, 3d showed better anti-bacterial and 3b, 3d, 3e, 3i showed better anti-fungal activity, when compared with ciprofloxacin and ketokonazole. Our present study makes it an interesting compound when compared to the present therapeutic agents and are considered the candidates to investigate further for the same purpose.

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